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New Understandings in Microbial (Bacterial) Evolution – a review

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Abstract

This review addresses the impact of the coming avalanche of genomic data and the convergence of fresh ideas on evolution on the pre-genetic concepts on bacterial evolution. The context is set with a brief historical account of the discovery of microbes and what Darwin wrote about them. The need to view bacterial evolution afresh in the 21st century is discussed. Current understanding of evolutionary forces and evolutionary mechanisms occurring in bacteria are outlined and secrets to the rapid evolution of bacteria are revealed. We conclude that the budding image of bacteria as gene-swapping entities stipulates a revision of such concepts as organism, species and evolution itself and propose a hypothesis that a bacterium is a 'composite entity' with a multiple decent of origin.

Keywords: microbial evolution; bacteria; evolution; evolutionary forces; evolutionary mechanisms

Finding the unseeable – what Darwin thought of microbes

In 1878, some 19 years after first publication of Darwin's "On Origin of Species", the French scientist Charles Sédillot proposed the word 'microbe' to refer to microscopic life (Bulloch 1938). However, predictions on the existence of minute organisms not visible to the naked eye long predate their discovery. As early as about 50 B.C. the Roman philosopher Titus Lucretius Carus speculated in his poem *De Rerum Natural* (On the Nature of the Universe) on the existence of invisible disease-causing entities, and in 36 B.C. Marcus Terentius Varro wrote that animals (*animalia quaedam minuta*) that cannot be followed by the eye were transferred through the air to other persons and caused serious illness (Cheesman 1964). We now think that Varro was referring to *Plasmodium*, the causative agent of malaria. Despite many centuries of philosophical and scientific speculation about their existence visual evidence for microbes was only first revealed to humans following improvements in microscopy during the 17th century, especially by the Dutch clothing merchant Antonie van Leeuwenhoek (van Leeuwenhoek 1684). Leeuwnhoek used the term 'animalcule' for the miniscule life forms he observed with his novel microscopes in various samples such as suspended teeth scrapings obtained from his friends and acquaintances. Much later the term 'infusoria' become popular to describe the abundant life forms seen in the microscopic world. However, apart from some interested scientists being aware of their existence there was little progress in knowledge of microbes during the next two centuries that followed their discovery. Whether these minute organisms were the cause or the products of biological or chemical processes were not clear to these early discoverers, and it was not until the beginnings of the science of microbiology by pioneers such as Louis Pasteur and Robert Koch in the mid-19th century that significant advances in understanding were made.

Studies on microbial biogeography also trace back to the days of Charles Darwin. It is commonly assumed that Darwin had nothing to say in his writings about microbes. However, this is not the case. He included microbes in his Beagle studies of the geographical distribution of organisms. Charles Darwin (1846) wrote "Generally the atmosphere is hazy; and this is caused by the falling of impalpably fine dust . . . I collected a little packet of this brown coloured fine dust... Professor Ehrenberg finds that this dust consists in great part of infusoria with siliceous shields, and of the siliceous tissue of plants. In five little packets which I sent him, he has ascertained no less than sixty-seven different organic forms! The infusoria, with the exception of two marine species, are all inhabitants of fresh-water. . . . It is, however, a very singular fact, that, although Professor Ehrenberg knows many species of infusoria peculiar to Africa, he finds none of these in the dust which I sent him. On the other hand, he finds in it two species which hitherto he knows as living only in South America. . . . After this fact one need not be surprised at the diffusion of the far lighter and smaller sporules of cryptogamic plants".

Darwin had made three very important evolutionary claims based on his various discussions about the microscopic world and these are critical arguments to understand his theory on evolution (O'Malley 2009).

1. All living entities, no matter how different they seem from animals and plants, undergo natural selection;
2. Microbes demonstrate that evolution is not a progression from simple to complex;
3. Microbes and their adaptive capacities are very important biological phenomena to understand the history of evolution on Earth.

The term microbes is used often to include both prokaryotic and eukaryotic microscopic organisms, in this paper we focus on one of the two domains of prokaryotic microorganisms – *Bacteria*, and in particular recent advances in our understanding of mechanisms affecting their evolution.

Microbiology in the 21st century – why we need to view bacterial evolution afresh

Although the majority of prokaryotic microbes (except for those causing serious diseases) have traditionally been considered as a minor part of life since their discovery in the seventeenth century, during the past few decades bacteria, as a general group of organisms, have rightly been given the greater attention they deserve. It is now widely accepted that microbes, both over time and space, have dominated a major portion of the history of life on earth. Microbes are the most ancient inhabitants of Earth. Some of the very old microfossils resembling bacteria found in Western Australia date from about 3.5 billion years ago (Schopf 1993). However, prokaryotic microbes are not only very ancient but also they are by far the most diverse and abundant forms of life on this planet. As a general type of organism, prokaryotes seem to be able to thrive on practically any substance, either organic or inorganic, that they can oxidise. They are ubiquitous in nature and occur not merely in fertile soils, fresh water ponds, ocean waters, plants, animals and the air but also in what is considered to be very extreme environments such as the super-heated hydrothermal vents, on the deep ocean floor, known as "black smokers" venting fluids at temperatures up to 350°C (Prieur *et al.* 1995), Antarctic ice floes (Lanoil *et al.* 2009), salt-saturated alkaline pools (Oren 2002), acid hot springs (Brierley & Brierley 1973), distilled water reservoirs (Moffet & Williams 1967) and even the upper atmosphere (Imshenetsky *et al.* 1979). The ability of these minute organisms to exploit such a cosmic range of environments is due to the tremendous breadth of genomic, and consequently physiological, variation found amongst them.

Until quite recently, our knowledge of prokaryotic organisms, and their metabolic diversity, was largely restricted to those bacteria commonly encountered everyday and that can be cultured in the laboratory under ambient conditions. However, since the advent of new molecular techniques and Metagenomics, which enabled sequencing of DNA extracted from communities of organisms, understanding of these microscopic forms have greatly expanded (Steele & Streit 2005). For example, a recent study by Fierer *et al.* (2007) used metagenomic analyses of a variety of samples of prairie, desert and rain forest soils to reveal that the average number of microbial species present in a single sample of soil probably surpasses the total number of microbial species that have been described and named to date (~7,500 archea and bacteria, ~80, 000 fungi and ~2, 000 viruses). Thus, estimates and understanding of microbial diversity have taken a quantum leap early in the 21st century and it is fair to say that we are still only just beginning to understand the various evolutionary processes that lead to such a tremendous degree of ecological, morphological and physiological diversity in microbes. The key metabolic processes that have evolved in microbes have had a great influence in the history of this planet. Just as one example, the evolution of anaerobic photosynthesis contributed to the accumulation of oxygen in the Earth's atmosphere (Margulis 1970). The dawn of the Metagenomic era is promising to reveal new novel biochemical processes that exist in microbes but that have been concealed to humans so far (Steel & Streit 2005). When discovered, these no doubt will potentially revolutionize some of the basic concepts not only in biology but also in other fields of science.

Dimension of bacteria – what we knew and what we now know

Aspects of the bacterial cell

Bacteria are unicellular organisms usually ranging between 0.2 – 10 μm in size. As with many aspects of microbiology there are striking exceptions to this range with the largest known prokaryotic organism, *Thiomargarita namibiensis*, having cells up to 750 μm in diameter (Shulz *et al.* 1999). However, the norm is that bacterial cells are microscopic in size and need to be magnified about 100 times to be easily observed. In Stainer & van Niel (1962), used the current knowledge, to describe the principal features that distinguish a prokaryotic cell from a eukaryotic cell as follows:

Absence of internal membranes which separate the resting nucleus from the cytoplasm, and isolate the enzymatic machinery of photosynthesis and respiration in specific organelles.

Nuclear division by fission, not by mitosis, a characteristic possibly related to the presence of a single structure that carries all the genetic information of the cell. (As discussed below, it is now known that bacteria do not always carry their genetic information in a single structure.)

The presence of a cell wall that contains a specific mucopeptide as its strengthening element. (it is now known that the nature of the mucopeptide can be quite diverse amongst different species of archaea and bacteria. Furthermore, some bacteria, such as mycoplasmas, do not have a cell wall.)

Two other significant features that differ prokaryotes from eukaryotes are that their cytoplasm is immobile (cytoplasmic streaming, pseudopodial movement, endocytosis and exocytosis are absent) and they contain distinct 70S type ribosomes. While mechanisms of gene transfer between individual cells and recombination of DNA can occur, these processes never involve gametogenesis and zygote formation in prokaryotes (Krieg 2005). The method of prokaryotic reproduction is always asexual, commonly by a process called binary fission where a single cell first replicates its genome and then divides into two cells. Certain genera of bacteria, namely members of *Bacillus*, *Clostridium* and *Sporosarcina* may have two phases to their life cycle, vegetative cells and endospores (Nicholson *et al.* 2000). Endospores are not reproductive structures but are dormant survival bodies that are produced from the vegetative cell by a process called sporulation. This process is triggered

when the living, vegetative cells of these bacterial genera are exposed to stressful or harsh environmental conditions. As one of the hardiest life forms known, the genera of bacteria that can produce endospores are able to withstand high temperatures, desiccation, freezing, excessive radiation, chemicals, the vacuum of space and many other environmental conditions that would easily kill eukaryotic organisms and vegetative bacterial cells (Nicholson *et al.* 2000). Being dormant survival structures endospores are metabolically inactive, somewhat like a plant seed, which are able to wait for the environment to again become favourable. Once environmental conditions improve, the endospore then germinates back into a living, vegetative bacterial cell that can grow and thrive.

Bacterial genome architecture

Bacterial genomes are compact structures and, in contrast to eukaryotic genomes, their protein-coding genes are virtually uninterrupted by introns. Therefore, the size of a bacterial genome is strongly linked with the number of genes it contains and as a result the evolutionary forces that act on individual genes have profound effects on overall genome architecture in these organisms (Kuo *et al.* 2009). The occurrence of repetitive and invert repeat regions of DNA sequence is a common feature of bacterial genomes (de Bruijn 1992; Shapiro 2005). Although it has been known for some time that in bacteria certain genes with a common function often occur in an operon (Jacob & Monod 1961), until recently, operons were thought to consist of both coding and non-coding, non-functional regions of DNA. Generally the term coding region is used to refer to DNA segments that contain the triplet genetic codes for amino acids that form polypeptides. It is now known that RNA and protein binding takes place on the so-called non-coding regions of an operon in order to fine-tune operon function (Shapiro 1999). These RNA and protein binding regions of operon DNA also contain specific codes of DNA sequence that interact with RNA or proteins but that work as quite different codes to the well-known triplet genetic code for amino acids. Thus the concept we now have for the existence of multiple genetic codes in living organisms has come to be (Shapiro 1999). In addition to the commonly known triplet genetic code for amino acids it is now known that quite a wide range of other genetic codes in bacterial genomes such as transcription codes, replication codes, DNA segregation codes and codes for control signals for the level of expression of a certain protein (Shapiro 1999). These findings also suggest that the commonly occurring repetitive regions in bacterial genome are not just "junk DNA" as previously thought but that these segments of DNA in fact contain these other genetic codes which are essential for the proper functioning of a bacterium (Shapiro 2005).

Multiple chromosomes, other replicons and their shapes

Until the advent and widespread application of genome-mapping studies, it was a common belief that all bacteria possessed a single circular chromosome. It is now know that this is not always the case – bacteria do not necessarily have a single chromosome nor are their chromosomes always circular. The presence of multiple chromosomes has been reported for a number of bacterial species; for example, two different chromosomes are known to exist in *Agrobacterium tumefaciens, Leptospira interrogans, Brucella melitensis* and *Rhodobacter sphaeroides* (Allardet-Servent *et al.* 1991, 1993; Choudhary *et al.* 1994; Zuerner *et al.* 1993) while three chromosomes are present in *Burkholderia cepacia* (Rodley *et al.* 1995). To date, two theories have been proposed for the evolution of multiple chromosomes in bacteria. One proposal is that the single chromosome splits into two or more smaller chromosomes. Evidence such a splitting of a single large chromosome to create multiple chromosomes has been reported for strains of *Bacillus cereus* (Carlson & Kolsto 1994). In this species the chromosomal splitting may be a relatively recent event in evolutionary terms, as contemporary strains of *B. cereus* with one or more chromosomes coexist. On the other hand the splitting of the chromosome in *L. interrogans* into two separate entities may have been a relatively ancient event as all strains of this species studied so far contain the two chromosomes (Zuerner *et al.* 1993).

The second mechanism proposed for generation of multiple chromosomes in bacteria is acquisition of the chromosome via lateral (horizontal) transfer of the DNA from another independent organism. Horizontal gene transfer is a very widespread process in bacteria that has played a very significant role in their evolution, as discussed further in the next section of this paper. Evidence for acquisition of a second chromosome via lateral (horizontal) transfer is found with *Rhizobium* sp. strain NGR234. This is a very promiscuous nitrogen fixing root nodule bacterial strain that can enter into symbiosis with legumes belonging to 112 genera representing all three sub-families of *Leguminosae* (Pueppke & Broughton 1999).

Given the existence of multiple chromosomes leads to the question – What are the benefits for bacteria of multiple chromosomes? Cole & Saint-Girons (1994) proposed that multiple chromosomes may have arisen in bacteria as an attempt by the cells to achieve faster overall rates of replication. It is well known that the capacity for rapid growth rates provides bacteria with strong advantages in competitive situations. However, the maintenance of multiple chromosomes across successive generations also requires the presence of an efficient system for chromosome partitioning, and this may cause a metabolic burden on the cell. These authors also suggested a possible reason for the evolution of multiple chromosomes in bacteria may be due to very strong selective pressure to maintain the second chromosome if it contains genes essential for bacterial growth or survival.

Until the 1980s the bacterial chromosome was also thought to only exist as a circular structure in the cytoplasm of the cell. However, during recent decades a range of interesting discoveries have been made relating to the diverse variety of shapes and sizes of bacterial chromosomes. Although the majority of known bacteria do contain a single circular chromosome, some bacterial chromosomes are linear molecules of DNA. A linear bacterial chromosome was first reported in the bacterial agent of Lime disease, *Borrelia burgdorferi* (Baril *et al.* 1989; Ferdows & Barbour 1989). Since then other bacteria with linear chromosomes have been found in the genera *Streptomyces* (Lin *et al.* 1993) and *Rhodococcus* (Crespi *et al.* 1992). In some bacteria the situation is even more

complex with both circular and linear chromosomes being present, such as the coexistence of both liner (2.1 Mb) and circular (3 Mb) chromosomes in *Agrobacterium tumefaciens*. This species also contains two circular plasmids (450 kb and 200 kb; Allardet-Servent *et al.* 1993). Plasmids are double-stranded DNA molecules containing genetic information that can exist and replicate independently of the chromosome, but may also integrate with the chromosome. Plasmids often are stable and inherited in bacteria, but the genetic information they contain is not always essential for their growth and reproduction. However, plasmids can contain genes, such as antibiotic or heavy metal resistance that enhance cell survival in adverse or stressful conditions and thus provide a selective advantage for the cells that contain them. As with bacterial chromosomes, plasmids are commonly circular molecules of DNA, but linear plasmids occur in *Mycobacterium xenopi, M. celatum* and *M. branderi* even though these species each have a circular chromosome (Picardeau & Vincent, 1997). Species of *Borrelia* contain both linear and circular plasmids (Barbour 1988).

Bacterial evolutionary mechanisms – secrets for rapid evolution

The evolutionary mechanisms of a bacterial genome can be divided into three broad functional categories (Brown *et al.* 2001). Two of these categories (local changes in DNA sequence and rearrangements of DNA) exert their evolutionary effects by changes to the existing DNA (commonly inherited from the parent cell) present in a cell. The third mechanism, (lateral (horizontal) gene transfer) operates via the introduction of new genes into the cell that can provide it with novel metabolic capabilities that it did not previously have.

The first category, local DNA sequence changes, includes all situations of small local changes in the DNA sequence such as occur with the substitution of a single nucleotide, the deletion or insertion of one or a few nucleotides, or the scrambling of a few nucleotides etc. These small local changes in DNA sequence are generally thought of as being responsible for the slow evolution of proteins, and such changes in genetic information are generally transferred vertically from mother to daughter cells through successive generations. However, local sequence changes can also contribute to rapid evolution if these small sequence changes occur for example in a regulatory gene for a particular metabolic pathway. This could result in the complete shut down of the pathway and consequently could have a great impact on the evolutionary path of the organism in question.

The second broad category of evolutionary mechanism is DNA rearrangements within the genome of the bacterial cell (Michel 1999). These are often a consequence of the movement of genes between various locations on the chromosome or even between plasmids and the chromosome. This general process of gene movement is called transposition, and the simplest form of such a mobile gene is known as an insertion sequence (IS). An IS is a specialised segment of DNA that only contains information for functions involved in the movement of the segment from one DNA site to another. One important outcome of transposition is a rearrangement of some of the DNA of the cell's genome, and depending on the nature of the genes affected there can be considerable effects on the organism itself, and the ecological niches it may occupy. There are a diverse range of mobile genetic elements now known to occur in bacteria (*e.g.* composite transposons, integrons, conjugative transposons), and the processes involved in their functioning within cells can cause substantial rearrangements of DNA as a consequence of events such as recombinational reshuffling, insertion sequence (IS) element related DNA inversion, deletion or partial genome duplication, site-specific recombination, transposition events, repetitive elements, correia elements and VCR elements (Buisine *et al.* 2002; Burrus *et al.* 2002; Burrus & Waldor 2004; Mazel *et al.* 1998; Chalmers & Blot 1999).

The third category of evolutionary mechanism is the main player in rapid evolution of bacterial genomes and this overall process is called lateral (horizontal) gene transfer (Gorgaten *et al.* 2002; Gorgaten & Townsend 2005). This overall process involves the transfer of DNA, and obviously the genetic information it contains, from one bacterial cell to another. There are three quite different ways in which a bacterium can gain DNA via lateral transfer from another cell. One process, called conjugation, is when there is DNA transfer from a donor cell to a recipient cell following direct cell-to-cell contact and involves bacterial plasmids in the process. The second way of gene transfer, known as transformation, occurs when a cell takes up exogenous DNA present in its environment and incorporates the foreign DNA into its genome. The third process of gene transfer, known as transduction, is the transfer of DNA between different bacteria by bacterial viruses, also known as bacteriophages. The evidence from many molecular-based studies of bacterial genomes is providing strong indications that these processes of genetic transfer are probably the most significant influence on rapid evolution in bacteria. Most bacteria share the ecological niches they inhabit with very diverse communities of organisms, and because of horizontal gene transfer they have potential access to a considerable portion of the community gene pool. There is growing evidence that within certain community gene pools, such as occur in soil populations of bacteria, there may be present what we would term "a flexible gene pool" that contains an 'assortment' of strain-specific genetic information that may provide additional properties enabling these bacterial species to adapt to special environments. The genes in this flexible gene pool are often carried on mobile genetic elements such as plasmids, genomic islands, *etc.*, and can easily transfer among bacterial strains, species or even genera facilitating their relatively rapid evolution.

Evolutionary forces – news and views

There is a large repertoire of sequenced bacterial genomes now available: the average size of a small bacterial genome is estimated to be about 0.5 Mb while the larger bacterial genomes are around 10 Mb (Kuo *et al.* 2009). However, the smallest bacterial genome sequenced so far is only 0.02 Mb containing just 29 genes (http://

img.jgi.doe.gov/cgi-bin/pub/main.cgi) and belongs to the parasitic bacterial species *Pasteuria nishizawae* (Noel *et al.* 2005). The largest bacterial genome sequenced to date is 13.6 Mb in size contains more than 13,000 genes (http://img.jgi.doe.gov/cgi-bin/pub/main.cgi) and is found in the filamentous, spore forming bacterial species *Ktedonobacter racemifer*, a soil saprophyte from Italy (Cavaletti *et al.* 2006). This is a somewhat unusual bacterium because spore formation and filamentous morphology are not the norm in bacteria. In fact these are the exceptions, and perhaps in part account for its large genome size.

Prior to the availability of genome sequencing technology, bacterial genome evolution was suggested by some researchers to have occurred by repeated events of genome doubling (Herdman, 1985; Wallace & Morowitz, 1973; Riley & Anilionis, 1978). However, comparative genome analysis of a large number of bacteria has shown that in some instances, closely related bacteria with genomes of similar size frequently contain dissimilar complements of genes, and arrangement of duplicated genes are not consistent across taxa (Huynen & Bork 1998; Jordan *et al.* 2001). Further, the genome sizes of individuals belonging to the same species have been found in some instances to be highly variable (Bergthorsson & Ochman 1995). Phylogenetic analysis has revealed that smaller bacterial genomes have often derived from larger genomes (Anderson & Kurland 1998). These new findings are contradictory to the earlier beliefs on bacterial genome evolution. Although the presence of duplications of DNA and consequential development of paralogous genes are evident in some bacterial genomes, there is increasingly mounting evidence that the processes of lateral transfer of genes between bacterial cells provides a primary route through which bacterial genomes acquire novel genes (Ochman *et al.* 2000; Lawrence & Retchles 2009; Hacker & Kaper 2002).

Although, it is clear that lateral gene transfer has been and continues to be a common process in bacteria, bacterial genomes seem to maintain their relatively small size and lack non-functional sequences. This tendency to maintain, rather than increase, genome size seems to result from a pervasive bias towards higher numbers of deletions than insertions in bacterial genomes (Mira *et al.* 2001). Bacterial genes are either lost as large deletions or inactivated via point or frame shift mutations and subsequently eroded from the genome when their selection is not strong. This deletional bias is a major force shaping bacterial genome size (Mira *et al.* 2001).

Darwinian selection and random genetic drift are two central forces that compete in driving evolutionary change according to modern evolutionary biology theories (Nei 2005). Genetic drift is the random change in the genetic composition of a population due to chance events causing participation of individuals in producing succeeding generations (Hallatschek *et al.* 2007). Mira *et al.* (2001) have proposed the following model for genome size evolution in bacteria: the processes of deletional bias and genetic drift cause genomes to contract in size; selection on gene function cause genomes to maintain DNA (genes); an increase in genome size depends on either duplication or the acquisition of exogenous DNA, but these events are only effective if the new genes confer some benefit to the organisms. This model can explain how pathogenic forms of some species may have a reduced genome size while free-living counterparts may have a considerably larger genome size (Ochman & Davalos 2006). Being sequestered inside a host, the pathogenic form may have restricted opportunities for gene uptake, and consequently lose pathways required for exogenous DNA incorporation as well as other genes that are not necessary for their growth and survival in a relatively stable niche inside a host organism. This leads to minimal genome sizes that are also known as resident genomes (Andersson and Kuland 1998). Powerful selection to minimize the material costs of cellular replication in some bacterial genomes in special niches or ecosystems is called genome streamlining (Giovannoni *et al.*. 2005). Genomic streamlining in microbes is a consequence of selection for metabolic efficiency (Lynch 2006).

The larger genomes of the free-living strains indicates more recurrent gaining of new genes which may be required for metabolic versatility or more efficient selection for the maintenance of weakly beneficial genes in the genome. Kuo *et al.* (2009) propose that variation in level of genetic drift coupled with the inherent bias towards deletions in bacterial genomes are the key forces that govern the evolution of genome complexity in bacteria. The interplay among mutation, natural selection, and genetic drift is responsible for the vast diversity observed for the microbial genomes (Kuo *et al.* 2009).

It is apparent that we are still only at the beginning of uncovering the nature of diversity amongst bacteria, and our understanding of the complexity of processes at interplay in microbial evolution is in its infancy. The widespread occurrence of horizontal gene transfer between bacteria is a highly significant characteristic distinguishing them from eukaryotes, but researchers have only recently become aware of how important its role is in enabling rapid evolution in bacteria. Science is currently within an exciting period of microbiology as molecular advances provides new insights into the elegant complexity of processes at play in microbial evolution, and it is clear the knowledge gained will significantly influence our understanding of evolutionary processes affecting all life on our planet.

References

Allardet-Servent A, Carles-Nurit M J, Bourg G, Michaux S & Ramuz M 1991 Physical map of the *Brucella melitensis* 16M chromosome. Journal of Bacteriology 173: 2219–2224.

Allardet-Servent A, Michaux-Charachon S, Jumas-Bilak E, Karayan L & Ramuz M 1993 Presence of one linear and one circular chromosome in the *Agrobacterium tumefaciens* C58 genome. Journal of Bacteriology 175: 7869–7874.

Andersson S G E & Kurl C G 1998 Reductive evolution of resident genomes. Trends in Microbiology 6: 263–268.

Barbour A G 1988 Plasmid analysis of *Borrelia burgdorferi*, the lyme disease agent. Journal of Clinical Microbiology 26: 475–478.

Baril C, Richaud C, Baranton G & Saint-Girons I 1989 Linear chromosome of *Borrelia burgdorferi*. Reseach in Microbiology 140: 507–516.

Bergthorsson U & Ochman H 1995 Heterogeneity of genome sizes among natural isolates of *Escherichia coli*. Journal of Bacteriology 177: 5784–5789.

Brierley C L & Brierley J A 1973 A chemoautotrophic and thermophilic microorganism isolated from an acid hot spring. Canadian Journal of Microbiology 19: 183–188.

Brown E W, LeClerc J E, Kotewicz M L & Cebula T A 2001 Three R's of bacterial evolution: how replication, repair, and recombination frame the origin of species. Environmental & Molecular Mutagenesis 38: 248–260.

Buisine N, Tang C M & Chalmers R 2002 Transposon-like Correia elements: structure, distribution and genetic exchange between pathogenic *Neisseria* sp. FEBS Letters 522: 52–58.

Burrus V, Pavlovic G, Decaris B & Guedon G 2002 Conjugative transposons: the tip of the iceberg. Molecular Microbiology 46: 601–610.

Burrus V & Waldor M K 2004 Shaping bacterial genomes with integrative and conjugative elements. Research in Microbiology 155: 376–386.

Bulloch W 1938 A History of Bacteriology. Oxford University Press, Oxford.

Carlson C R & Kolsto A B 1994 A small (2.4 Mb) *Bacillus cereus* chromosome corresponds to a conserved region of a larger (5.3 Mb) *Bacillus cereus* chromosome. Molecular Microbiology 13: 161–169.

Cavaletti L, Monciardini P, Bamonte R, Schumann P, Rohde M, Sosio M & Donadio S 2006 New lineage of filamentous, spore-forming, gram-positive bacteria from soil. Applied & Environmental Microbiology 72: 4360–4369.

Chalmers R & Blot M 1999 Insertion sequences and transposons. *In*: Organization of the prokaryotic genome. R L Charlebois (ed). American society for microbiology, Washington DC, pp.151–170.

Cheesman D F 1964 Varro and the small beasts: a bimillennium for microbiologists. Nature 203: 911–912.

Choudhary M, Mackenzie C, Nereng K, Sodergren E, Weinstock G M & Kaplan S 1994 Multiple chromosomes in bacteria: structure and function of chromosome II of *Rhodobacter sphaeroides* 2.4.1T. Journal of Bacteriology 176: 7694–7702.

Cole S T & Saint-Girons I 1994 Bacterial genomics. FEMS Microbiology Reviews 14: 139–160.

Crespi M, Messens E, Caplan A B, Van Montagu M & Desomer J 1992 Fascination induction by the phytopathogen *Rhodococcus fascians* depends upon a linear plasmid encoding a cytokinin synthase gene. EMBO Journal 11: 795–804.

Darwin C R 1846 An account of the fine dust which often falls on vessels in the Atlantic ocean. Quarterly Journal of the Geological Society of London 2: 26–30.

de Bruijn F J 1992 Use of Repetitive (Repetitive Extragenic Palindromic and Enterobacterial Repetitive Intergeneric Consensus) sequences and the polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* isolates and other soil bacteria. Applied & Environmental Microbiology 58: 2180–2187.

Ferdows M S & Barbour A G 1989 Megabase-sized linear DNA in the bacterium the *Borrelia burgdorferi*, lyme disease agent. USA 86: 5969–5973.

Fierer N, Breitbart M, Nulton J, Salamon P, Lozupone C, Jones R, Robeson M, Edwards R A, Felts B, Rayhawk S, Knight R, Rohwer F & Jackson R B 2007 Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of bacteria, archaea, fungi, and viruses in Soil. Applied & Environmental Microbiology 73: 7059–7066.

Giovannoni S J, Tripp H J, Givan S, Podar M, Vergin K L, Baptista D, Bibbs L, Eads J, Richardson T H, Noordewier M, Rappé M S, Short J M, Carrington J C & Mathur E J 2005 Genome streamlining in a cosmopolitan oceanic bacterium. Science 309: 1242–1245.

Gogarten J P, Doolittle W F & Lawrence J G 2002 Prokaryotic evolution in light of gene transfer. Molecular Biology & Evolution 19: 2226–2238.

Gogarten J P & Townsend J P 2005 Horizontal gene transfer, genome innovation and evolution. Nature Reviews Microbiology 3: 679–687.

Hacker J & Kaper J B 2002 Pathogenicity Islands and the Evolution of Pathogenic Microbes. Springer, New York.

Hallatschek O, Hersen P, Ramaanathun S & Nelson D R 2007 Genetic drift at expanding frontiers promotes gene segregation. USA 104: 19926–19930.

Herdman M 1985 The evolution of bacterial genomes. *In*: The Evolution of Genome Size. T Cavalier-Smith (ed). John Wiley & Sons, pp 37–68.

Huynen M & Bork P 1998 Measuring genome evolution. USA 95: 5849–5856.

Imshenetsky A A, Lysenko S V & Lach S P 1979 Microorganisms of the upper layer of the atmosphere and the protective role of their cell pigments. Life Science & Space Research 17: 105–110.

Jacob F & Monod J 1961 Genetic regulatory mechanisms in the synthesis of proteins. Journal of Molecular Biology 3: 318–356.

Jordan I K, Makarova K S, Spouge J L, Wolf Y I & Koonin E V 2001 Lineage-specific gene expansions in bacterial and archaeal genomes. Genome Research 11: 555–565.

Krieg N R 2005 Prokaryotic domains. *In*: Bergey's manual of systematic bacteriology. G M Garrity (ed). Springer-Verlag, New York.

Kuo C H, Moran N A & Ochman H 2009 The consequences of genetic drift for bacterial genome complexity. Genome Research 19: 1450–1454.

Lanoil B, Skidmore M, Priscu J C, Han S, Foo W, Vogel S W, Tulaczyk S & Engelhrdt H 2009 Bacteria beneath the West Antarctic ice sheet. Environmental Microbiology 11: 609–615.

Lawrence J G & Retchless A C 2009 The interplay of homologous recombination and horizontal gene transfer in bacterial speciation. Methods in Molecular Biology 532: 29–53.

Lin Y S, Kieser H M, Hopwood D A & Chen C W 1993 The chromosomal DNA of *Streptomyces lividans* 66 is linear. Molecular Microbiology 10: 923–933.

Lynch M 2006 Streamlining and simplification of microbial genome architecture. Annual Reviews in Microbiology 60: 327–349.

Margulis L 1970 Origin of Eukaryotic Cells. Yale University Press, Connecticut.

Mazel D, Dychinco B, Webb V A & Davies J 1998 A distinctive class of integron in the *Vibrio cholerae* genome. Science 280: 605–608.

Michel B 1999 Illegitimate recombination in bacteria. *In*: Organization of the prokaryotic genome. R Charlebois (ed). American society for Microbiology, Washington DC, pp 129–150.

Mira A, Ochman H & Moran N A 2001 Deletional bias and the evolution of bacterial genomes. Trends in Genetics 17: 589–596.

Moffet H L & Williams T 1967 Bacteria recovered from distilled water and inhalation therapy equipment. 114: 7–12.

Nei M 2005 Selectionism and neutralism in molecular evolution. Molecular Biology and Evolution 22: 2318–2342.

Nicholson W L, Munakata N, Horneck G, Melosh H J & Setlow P 2000 Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiology & Molecular Biology Reviews 64: 548–572.

Noel G R, Atibalentja N & Domier L L 2005 Emended description of *Pasteuria nishizawae*. International Journal of Systematic & Evolutionary Microbiology 55: 1681–1685.

O'Malley M A 2009 What did Darwin say about microbes, and how did microbiology respond? Trends in Microbiology 17: 341–347.

Ochman H, Lawrence J G & Groisman E A 2000 Lateral gene transfer and the nature of bacterial innovation. Nature 405: 299–304.

Ochman H, & Davalos L M 2006 The nature and dynamics of bacterial genomes. Science 311 (5768): 1730–1733.

Oren A 2002 Molecular ecology of extremely halophilic Archaea and Bacteria. FEMS Microbiology Ecology 39: 1–7.

Picardeau M & Vincent V 1997 Characterization of large linear plasmids in mycobacteria. Journal of Bacteriology 179: 2753–2756.

Prieur D, Erauso G & Jeanthon C 1995 Hyperthermophilic life at deep-sea hydrothermal vents. Planet and Space Science 43: 115–122.

Pueppke SG & Broughton W J 1999 *Rhizobium* sp. strain NGR234 and *R. fredii* USDA257 share exceptionally broad, nested host ranges [Review]. Molecular Plant-Microbe Interactions 12: 293–318.

Riley M & Anilionis A 1978 Evolution of the bacterial genome. Annual Reviews in Microbiology 32: 519–560.

Rodley P D, Romling U & Tummler B 1995 A physical genome map of the *Burkholderia cepacia* type strain. Molecular Microbiology 17: 57–67.

Schopf J W 1993 Microfossils of the early archean apex chert: new evidence of the antiquity of life. Science 260: 640–647.

Shapiro J A 1999 Genome system architecture and natural genetic engineering in evolution. *In*: Molecular Strategies for Biological Evolution. L Caporale (ed). The New York Academy of Sciences, New York, pp 23–35.

Shapiro J A 2005 A 21st century view of evolution: genome system architecture, repetitive DNA, and natural genetic engineering. Gene 345: 91–100.

Shulz H N, Brinkhoff T, Ferdelman T G, Marine M H, Teske A & Jorgensen B B 1999 Dense populations of a giant sulphur bacterium in Namibian shelf sediments. Science 284: 493–495.

Stanier R Y & van Niel C B 1962 The concept of a bacterium. Archives of Microbiology 42: 17–35.

Steele H L & Streit W R 2005 Metagenomics: Advances in ecology and biotechnology. FEMS Microbiology Letters 247: 105–111.

van Leeuwenhoek A 1684 Microscopical observations about animals in the scurf of the teeth. Philosophical Transactions of the Royal Society of London. 14: 568–574.

Wallace D C & Morowitz H J 1973 Genome size and evolution. Chromosoma 40: 121–126.

Zuerner R L, Herrmann J L & Saint-Girons I 1993 Comparison of genetic maps of two *Leptospira interrogans* serovars provides evidence for two chromosomes and intraspecies heterogeneity. Journal of Bacteriology 175: 5445–5451.